Shoring up DNA methylation and H3K27me3 domain demarcation at developmental genes

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Short summary

Mutual antagonism between DNA methylation and H3K27me3 histone methylation suggests a dynamic crosstalk between these epigenetic marks that could help ensure correct gene expression programs. Work from Manzo and colleagues now shows that an isoform of de novo DNA methyltransferase DNMT3A provides specificity in the system by depositing DNA methylation at adjacent 'shores' of hypomethylated bivalent CpG islands (CGI) in mouse embryonic stem cells (mESCs). DNMT3A1-directed methylation appears to be instructive in maintaining the H3K27me3 profile at the hypomethylated bivalent CGI promoters of developmentally important genes.

DNA methylation represents a primary epigenetic mechanism in vertebrate and plant genomes for locking chromatin in a state that can repress the regulatory activity of DNA sequences associated with promoters and enhancers (Deichmann, 2016), maintain stable inheritance of imprinted genes and X-inactivation states in mammals, restrict tissue specific gene expression (Reddington et al, 2013a), and act as a general mechanism to silence intergenic transposons (Bestor et al, 2015). New evidence has emerged of the fine control of epigenetic organization at regulatory regions prior to differentiation.

Until recently, our conceptual view of DNA methylation in mammals was of a highly stable modification resulting from the combined action of maintenance (DNMT1) and *de novo* DNA methyltransferases (DNMT3A and DNMT3B), which perpetuate committed epigenetic states (Deichmann, 2016). However, this cosy interpretation has been challenged on a number of fronts: (i) the discovery of new DNA modifications in addition to 5-methylcytosine (5mC) that are potentially part of a DNA demethylation pathway, (ii) the effect of global DNA methylation patterns on the distribution of repressive chromatin mark histone H3 lysine 27 tri-methylation (H3K27me3), which revealed interdependence of epigenetic marks and, (iii) the lesser dependence of pluripotent embryonic stem cells (ESCs), at least in the case of mice, on the repression function of DNA methylation to maintain expression states and transposon silencing (An et al, 2017; Bestor et al, 2015; Marks & Stunnenberg, 2014). From the point of view of mouse development, DNA methylation patterns are established during a dynamic reprogramming phase so that they can contribute to Gene Regulatory Networks (GRNs) that, in transitioning to differentiated cells, rely on the repressive effect of DNA at specific regulatory sequences (promoters, enhancers, Transposon-LTRs) to maintain developmentally induced silencing and normal H3K27me3 profiles (Bestor et al, 2015; Reddington et al, 2013b).

The potential link between DNA methylation and appropriate GRN activation in differentiated cells implies additional specificity in the targeting of DNA methylation to regulatory sequences. Antagonism between DNA methylation and H3K27me3 suggests that a dynamic crosstalk between these epigenetic marks could facilitate correct gene expression programs (Reddington et al, 2013a). In an elegant and comprehensive study, Manzo and colleagues examined the different roles of DNMT3 *de novo* methyltransferase isoforms in establishing DNA methylation patterns in an mESC model and derived neuronal cells (Manzo et al, 2017). Using ΩAGE sequencing data-sets they found that the short isoform DNMT3A2, lacking an N-terminal extension, is mainly expressed during early development, whereas a longer DNMT3A1 isoform is ubiquitously expressed in the majority of samples. Through tagging of the individual DNMT3 isoforms expressed from the same heterologous genomic site at similar levels in mESCs, they obtained comparative binding profiles for DNMT3A1, DNMT3A2 and DNMT3B1 revealing several differences: (i) lack of binding of DNMT3A isoforms to actively transcribed gene bodies occupied by DNMT3B (Baubec et al, 2015) (ii) differential localization of the isoforms at numerous genomic sites (e.g. the Hoxb locus) and, (iii) the preferential localization of DNMT3A1 to genomic sites enriched for H3K27me3, which was not observed for the DNMT3A2 or DNMT3B profiles. In fact, DNMT3A1 occurs mainly at hypomethylated bivalent CpG islands (CGI), which are associated with silent genes that are marked by both H3K27me3 (repressed) and H3K4me3 (active marks) in ESCs. The presence of H3K4me3 excludes DNMT3A1 from the centre of these hypomethylated bivalent CGIs, confining its localization at the upstream and downstream CGI 'shores' that can be differentially methylated during development and in cancer. DNMT3B is not present at CGI shores, while DNMT3A2 is present to a minor extent. Of interest is that DNMT3A1, but not DNMT3A2, follows H3K27me3 dynamics, and preferentially relocates to the vicinity of H3K27me3 during neuronal differentiation; this relocation of DNMT3A1 to Polycomb-regulated CGIs is dependent on its N-terminal region.

Previous work has shown an association between TET1 (a methylcytosine dioxygenase), Polycomb group proteins, and the presence of 5-hydroxymethylcytosine (5hmC) at bivalent CGI; in line with the presence of DNMT3A1 at CGIs, this provides the 5mC substrate for 5hmC acquisition. The enzymatic activity of DNTMT3A1 could be important for regulating H3K27me3 deposition at bivalent CGIs (Williams et al, 2011). In Dnmt3a/3b KO or Dnmt-triple-KO ES cells, H3K27me3 at bivalent CGIs is reduced but re-expression of DNMT3A1 in these KO ES cell lines preferentially re-targets DNA methylation to bivalent CGI shores and can promote re-acquisition of H3K27me3 (King et al, 2016). These results strongly suggest that DNMT3A1 is responsible for sustaining methylcytosine at bivalent CGIs shores, which links with H3K27me3 domain demarcation and potentially subsequent regulation of Polycomb target genes (Figure 1).

It has been suggested that mESCs represent a cell state that exhibits little dependence on repressive epigenetic systems as evidenced by the viability of Dnmt and Polycomb mutants. However, this new publication indicates that the interplay between DNA and Polycomb group protein machineries is important for setting up epigenetic states during mouse embryogenesis that will be utilised in differentiating cells to reinforce differential gene expression (Bestor et al, 2015). It should also be noted that deletion of DNMT1 resulted in rapid cell death in human ESCs, which may suggest differences in epigenetic dependencies between species. It is not clear for instance if global DNA methylation reprogramming is a common feature for early mammalian development, as sheep embryos appear to maintain high levels of DNA methylation throughout early embryogenesis (Beaujean et al, 2004; Liao et al, 2015). This suggests that the role of epigenetics in regulating early

development needs a more nuanced and perhaps context-dependent view. The newly revealed organisational detail of epigenetic states clarifies the bivalent promoter set up prior to cell differentiation and opens the way for other epigenetic micro-contexts providing new interpretations of long standing epigenetic paradigms.

Figure 1 legend: *DNA methylation mediated by the de novo methyltransferase DNMT3A1 influences Polycomb targeting at bivalent hypomethylated CpG islands (CGI). PRC2 targeting has been linked to unmethylated transcriptionally inactive CGIs. CpG methylation restrains the PRC2-catalysed H3K27me3 histone mark, which can exhibit mutual antagonism within CGIs of mammalian genomes. Based on Figure EV2 from Manzo et al (this issue).*

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